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ADDITION OF Ni AND Mo AS AN EFFORT TO INCREASE ETHANOL IN PALM OIL WASTEWATER TREATMENT

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ABSTRACT: Ethanol is an alternative energy that can be considered to replace energy sources derived from fossils. Ethanol can be produced at the acidogenesis stage of the anaerobic conversion process of palm oil mill effluent wastewater. The efficiency of bioethanol formation is influenced by several factors, including the presence of micronutrients such as molybdenum and nickel. The purpose of this study was to determine the effect of the presence of molybdenum and nickel in the anaerobic wastewater treatment process on the formation of ethanol. Palm oil mill effluent was used as a substrate which was added with Mo with a concentration of 1 and 3 mg/L and Ni of 0.25 and 0.5 mg/L. Mixed culture bacteria as biomass was used in the reactor. CBR reactor is used with an operational time of 72 hours. The reactor performance was investigated by measuring ethanol production, volatile fatty acids, VSS, pH, and soluble COD. It was found that in the reactor with the addition of variations of Mo 3 mg/L and Ni of 0.25 mg/L ethanol production was higher than the other reactor about 578.44 mgCOD/L, while the higher COD removal occurs in the reactor with the addition of Mo and Ni variations of 1 mg/L and 0.25 mg/L respectively, which was 23.4%. These results show that the anaerobic product formation was influenced by the presence of molybdenum and nickel.

Keywords: Anaerobic processes, Ethanol, Palm oil mill effluent, Volatile fatty acids.

1. INTRODUCTION

The utilization of renewable energy is an alternative that can be developed to replace dependence on energy sources originating from fossils. Currently, the main energy sources come from fossil fuels consisting of petroleum (35%), coal (29%), and natural gas (24%) [1]. There are at least three main problems with the use of fossil fuels, including the depletion of known petroleum reserves, the instability of world oil prices due to a higher demand rate from oil production itself, and the emergence of greenhouse gases as a result of burning fossil fuels.

Ethanol is an alternative energy that can substitute fossil fuels. The advantages of ethanol used as fuel were supporting a sustainable economy by reducing the use of fossil fuels, reducing CO_2 accumulation, and leading to zero net CO_2 output into the atmosphere [2]. Ethanol production capacity in 2005 and 2006 was about 45 and 49 billion liters respectively and it increased in 2015 by more than 115 billion liters [3].

The previous research indicated that high organic content wastewater can be used as a substrate for ethanol production using anaerobic processes. Palm oil mill effluent (POME) had a high concentration of organic, such as carbohydrate, protein, nitrogen compounds, oil and grease, high mineral content, and other organic compounds, such as cellulose, hemicellulose, and starch [4]. BOD and soluble COD concentration of POME were about 12,450 mg/L and 17,300 mg/L respectively [5]. Bioconversion of the high organic content wastewater can be used for biofuels production such as ethanol, hydrogen, methanol, and ethanol [6]. The anaerobic process pathway proceeds in four stages, that are hydrolysis, acidogenesis, acetogenesis, and methanogenesis [7]. The ethanol formation process is formed in the acetyl-CoA/Wood-Ljungdahl pathway for the production of acetyl-CoA, a precursor of cellular biomass, acetate, and ethanol. Key enzymes of this pathway include format dehydrogenase (FDH), bifunctional carbon monoxide dehydrogenase/ acetyl CoA synthase (CODH/ACS), and hydrogenase (H_2ase), all of which are metalloenzymes [8].

Ethanol and other intermediate products are formed during the acidogenic phase. The anaerobic fermentation process involves cell metabolism influenced by micronutrients, such as metal ions even though the amount of it may be very low [9]. Some research has been reported the influence of metal addition to ethanol production. The addition of iron (II) concentration of 200 mg/L produced ethanol equal to the control (without iron addition), while the addition of iron concentration of 400 mg/L produced ethanol 122 times lower than control and 600 mg/L of iron (II) could not produce the ethanol [10]. The combination of iron (II) and other metals also influenced ethanol production.

For another combination of iron (II) and magnesium (II) with the concentration are 0.5 mg/L-Fe + 0.5 mg/L-Mg; 1.5 mg/L-Fe + 0.5 mg/L-Mg and 2.5 mg/L-Fe + 0.5 mg/L-Mg produced ethanol with the concentration are 463.7; 572.3 and 689.7 mg/L respectively [5]. The experimental efficiency of ethanol yield under optimal conditions enhances the efficiency of ethanol yield by 5.63% by the addition of Mn^{2+} [9]. The addition of combination Mn^{2+} and Mg^{2+} can increase ethanol concentration from 93.45 g/L to 120.58 g/L sweet sorghum juice media [11]. Ethanol concentration increases from 64,53 g/L to 85,62 g/L in the addition of Cu^{2+} and Mn^{2+} from cane molasses. Cu^{2+} acts as a cofactor of some enzymes, such as cytochrome c oxidase, Cu, Zn-superoxide dismutase. Regarding Mn^{2+}, it is important in the metabolism of *S. cerevisiae* as a part of some enzymes relating to ethanol fermentation such as pyruvate carboxylase [11]. Mg^{2+} involves in physiological function, growth, metabolism, and enzyme activity. Mg^{2+} had a positive effect on ethanol production. Mg^{2+} reduces the proton, especially anion permeability of the plasma membrane by interacting with membrane phospholipids, resulting in stabilization of the membrane bilayer. Therefore, it relates to the improvement of ethanol tolerance of microorganisms. According to Lettinga [12], the chemical composition of the methanogenic microorganisms is Fe, Ni, Co, Mo, Zn, Mn, and Cu.

This research aimed to determine the effect of metal ion (Ni and Mo) addition on the ethanol formation from the POME treatment under anaerobic conditions. Metal ions have been found to maintain structural integrity and functionality of intracellular organelles, induce cell-cell interaction such as flocculation, govern gene expression and nutrient uptake mechanisms, activate arrays of enzymes intimately involved in metabolism, and also, act as stress-protectants. Metal ions can affect the rate of glycolysis and conversion of pyruvate to ethanol and other volatile organic acids [13], increased fermentability and ethanol yield, improved yeast viability and vitality [14]. The products of the acidogenesis phase indicate a shifting of the fermentation pathway. This shifting was interfered with by the enzyme availability. The enzyme that contains iron and zinc that are usually available in Gram-negative bacteria is the main factor for ethanol production [15]. Alcohol dehydrogenase enzyme (ADH) was the main enzyme for ethanol production. Iron addition increased the activity of the alcohol dehydrogenase enzyme therefore the ethanol production could be increased [16].

2. RESEARCH SIGNIFICANCE

The novelty and originality of this research is the effect of the metal addition to increasing the production of ethanol in the anaerobic processing of palm oil mill effluent wastewater. The metal addition to the wastewater can be used as a basis for consideration in a strategy to control the formation of bioenergy towards such as ethanol. It is hoped that this research can make a scientific contribution and can provide information related to the use of palm oil mill effluent wastewater as a substrate for the formation of ethanol as alternative energy.

3. MATERIAL AND METHODS

3.1. Wastewater and Bioreactor

This research was conducted on a laboratory scale. The wastewater, for substrate, was collected from the palm oil industry, PT Condong Garut, Indonesia. Biomass was taken from the sludge of pome mixed with cow rumen with the ratio of 50:50 (v/v) and acclimated to the wastewater. The concentration of biomass in terms of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solid (MLVSS), were about 6.34 g ss/L and 4.72 g vss/L respectively.

Anaerobic batch reactor with the working volume of 5l was operated with the flushing n_2 1L/min for the first 24 h for purging O_2 in the headspace and continued with internal biogas circulation until 72 h (Fg.1).

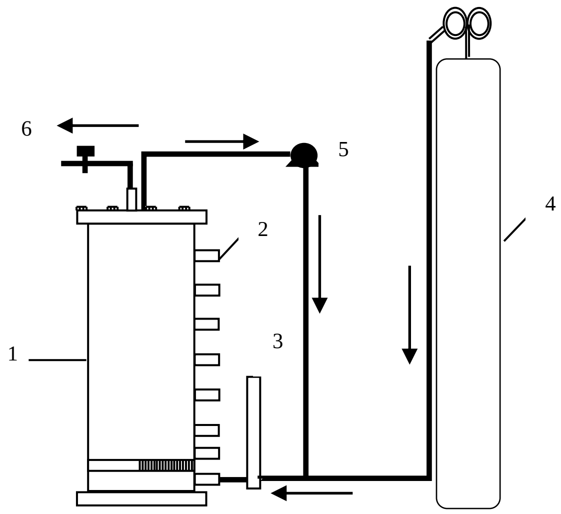

Fig. 1 Anaerobic batch reactor configuration: (1) Circulating bed reactor (CBR); (2) sampling port; (3) flow meter; (4) nitrogen gas (N_2); (5) recirculation pump; (6) gas ventilation.

The reactor was operated at room temperature (25ºC ± 2ºC). In the beginning, pH was adjusted to 6.5-7.0. Reactor filled by POME as a substrate and biomass with the ratio 4:1 (v/v). The concentration of the nickel addition was 0.25; 0.50 mg/L and 0 mg/L as a control and the concentration of molybdenum addition were 1; 3 mg/L and 0 mg/L as a control. $NiSO_4 6H_2O$ (p.a) was used as a source of nickel and $(NH_4)_6Mo_7O_{24}4H_2O$ as a source of molybdenum. The addition of the metal ion was supplemented into POME at concentrations indicated in Table 1. Samples were collected every 6 h and analyzed for soluble COD, pH, VSS, ethanol, and volatile fatty acid.

Table 1 Experimental design for study effect of trace metal on ethanol production

Run	Ni (mg/L)	Mo (mg/L)	Notes
1		0	Control
2	0	1	Reactor A
3		3	Reactor B
4		0	Reactor C
5	0.25	1	Reactor D
6		3	Reactor E
7		0	Reactor F
8	0.5	1	Reactor G
9		3	Reactor H

3.2. Analytical Method

Soluble COD, pH, and VSS were analyzed refer to Standard Method for the Examination Water and Wastewater 23rd edition 2017. Volatile fatty acids were analyzed using HPLC type Hitachi Interface D-7000 HSM (UV-Vis detector Hitachi L-7400 and L-7100 Hitachi pump) with analytical column Aminex HPX-87H, and ethanol was analyzed using GC Shimadzu 17-A with column Analytic DB-Wax (propylene glycol).

3.3. Calculation

Determination of the optimum conditions based on the efficiency and the formation of ethanol and a few other variables. The units of each product are uniformized by using the following equation [17].

Acidogenic product equation:

$$COD_{theoritical}=8(4x+y-2z)/(12x+y+16z)gCOD.g^{-1}CxHyOz \quad (1)$$

Note: x = C; y =H and z = O

Degree of acidification (DA):

$$\mathbf{DA} = \frac{[\mathbf{TAV}]\ (\frac{\mathbf{mgCOD}}{\mathbf{L}})}{\mathbf{influent\ of\ soluble\ COD}\ (\frac{\mathbf{mgCOD}}{\mathbf{L}})} \quad (2)$$

Degree of ethanofication (DE):

$$\mathbf{DE} = \frac{[\mathbf{Ethanol}]\ (\frac{\mathbf{mgCOD}}{\mathbf{L}})}{\mathbf{influent\ of\ soluble\ COD}\ (\frac{\mathbf{mgCOD}}{\mathbf{L}})} \quad (3)$$

Significant differences in ethanol production were analyzed by one-way ANOVA using IBM SPSS 26, with H0 there is no significant difference in adding trace elements while in H1 there is a significant difference in adding trace elements.

4. RESULTS AND DISCUSSION

4.1. Characteristics of Palm Oil Mill Effluent (POME)

The characteristic of POME which was used for ethanol production was presented in Table 2. POME had a high concentration of organic material, as soluble COD and BOD was a concentration of 18,433 mg/L and 14,500 mg/L respectively. Both COD and BOD reflect the high concentration of organic and it will potentially environmental problem for any untreated discharged. POME also contained macro and micronutrients such as nitrogen, potassium, magnesium, calcium, cadmium, copper, chromium, and iron. Carbohydrates, lipids, and minerals are found in POME [18]. POME is one of the wastewaters which difficult to treat due to its massive and unproper treatment [19]. The processing carried out at this time is still unable to remove the hazardous compounds contained in palm oil mill effluent [20]–[22]. Currently, most of the processes that are carried out to treat POME are in open ponds which are relatively low cost and easy to do. However, this processing has the potential to pollute the environment, for example, the emergence of the greenhouse gas effect [23]–[25], also takes a fairly large area [26].

Table 2 Characteristic of palm oil mill effluent

Parameter	Conc
Total COD	30367
BOD	14500
Soluble COD	18433
pH	4,3
Oil and grease	630
Ammonia (NH_3)	26,77
Nitrogen total	265,67
Total suspended solids (TSS)	6200
Volatile suspended solids (VSS)	3967
Ethanol	44,4
Volatile fatty acids (VFA’s)	566,67
Iron (Fe)	14,96
Manganese (Mn)	2,09
Molybdenum (Mo)	< 0,001
Nickel (Ni)	0,025
Cooper (Cu)	0,073
Zinc (Zn)	0,051

Note: All units in mg/L, pH without unit

Ethanol can be produced through the proper processing of high concentrations of organic wastewaters. It means that POME could be used as a substrate for ethanol production under anaerobic conditions. The products produced in this processing are strongly influenced by the conditions of the processing itself.

4.2. Effects of Trace Metals on The Ethanol Production

In this study, nickel and molybdenum were added to the reactor with different concentration variations. These trace elements were added individually or a combination of several concentrations, with one control reactor without adding trace elements. This research was carried out in 9 reactors with the following identities: control reactor (without the addition of micronutrients); reactor A (0 mg/L-Ni + 1 mg/L-Mo); reactor B (0 mg/L-Ni + 3 mg/L-Mo); reactor C (0.25 mg/L-Ni + 0 mg/L-Mo); reactor D (0.25 mg/L-Ni + 1 mg/L-Mo); reactor E (0.25 mg/L-Ni + 3 mg/L-Mo); reactor F (0.5 mg/L-Ni + 0 mg/L-Mo); reactor G (0.5 mg/L-Ni + 1 mg/L-Mo); and reactor H (0.5 mg/L-Ni + 3 mg/L-Mo).

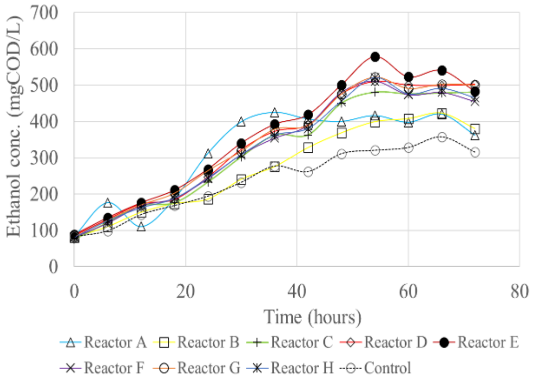

Fig. 2 Ethanol concentration in the reactor during the processes

In **Error! Reference source not found.**, it can be seen that an increase in the concentration of ethanol occurred in almost all reactors from the beginning to the 54th hour. After 54 hours, the ethanol concentration tended to be stable and even decreased. Compared to the control, the increment of Ni and Mo addition improved ethanol production. The experiment indicated that the concentration of Ni and Mo addition influenced the lag and the exponential phase of ethanol production. In the reactor with the addition of 0.25 mg/L-Ni + 3 mg/L-Mo (reactor E) ethanol with a concentration of 578.44 mgCOD/L can be produced, this value is the largest when compared to ethanol produced in other reactors. Reactor G and H with the addition 0.5 mg/L-Ni + 1 mg/L-Mo and 0.5 mg/L-Ni + 3 mg/L-Mo produced almost similar ethanol at 521.68 mgCOD/L and 520.88 mgCOD/L. Reactors A and B which only added 1 and 3 mg/L of Mo (without Ni addition) respectively, could produce the ethanol with the concentration 416.78 mgCOD/L and 399.39 mgCOD/L respectively. The addition of trace metal at the optimum concentration was increased ethanol production by about 63.6%. In some previous studies, it can also be found that the presence of micronutrients can increase ethanol production. Demirel and Scherer [27] reported that the addition of trace elements such as Fe, Mo, Ni, Co, etc. can have a positive influence on the anaerobic process, which otherwise does not substrate. A trace element is an essential part of enzymes as a co-factor in the anaerobic process stage which directly affects the performance of microbes and can increase the efficiency in the anaerobic system [28].

Fig. 3 Anaerobic reactor during the processes

A significant test was carried out to see the effect of adding trace elements to the formation of ethanol. The stages in the ANOVA test consist of normality test, homogeneity test, and ANOVA test. In the normality and homogeneity tests, the decision-making criteria are based on the significance value (Sig.) which if the Sig. > 0.05 then the data is normally distributed (homogeneous in the homogeneity test), whereas if the value of Sig. < 0.05 then the data is not normally distributed (not homogeneous in the homogeneity test). The results of the normality test can be seen in Table 3. Based on the results of the normality test, the significance value of all reactors was more than 0.05. For example, in the control reactor, the significance value is 0.109 which means more than 0.05. The same thing happened to other reactors. This shows that the data is normally distributed.

Table 3 Test of Normality (output from the SPSS)

Tests of Normality

	Reactor_code	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Ethanol Conc	Control	,365	3	.	,798	3	,109
	Reactor A	,260	3	.	,958	3	,607
	Reactor B	,351	3	.	,828	3	,183
	Reactor C	,218	3	.	,987	3	,786
	Reactor D	,291	3	.	,925	3	,471
	Reactor E	,256	3	.	,962	3	,625
	Reactor F	,175	3	.	1,000	3	,991
	Reactor G	,182	3	.	,999	3	,936
	Reactor H	,236	3	.	,977	3	,711

a. Lilliefors Significance Correction

Furthermore, a homogeneity test is conducted to see whether the resulting data is homogeneous or not. The results of the homogeneity test using SPSS can be seen in Table 4. Based on the results of the homogeneity test for the average value, the significance value is 0.546. This means the value of Sig. > 0.05 which means that the data is homogeneous.

Table 4 Test of Homogeneity (output from the SPSS)

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Ethanol Conc	Based on Mean	,887	8	18	,546
	Based on Median	,284	8	18	,963
	Based on Median and with adjusted df	,284	8	11,323	,958
	Based on trimmed mean	,832	8	18	,587

The next step is the ANOVA test to compare the difference in the average ethanol produced from each reactor. The basis for making decisions on this ANOVA test are:

1. If the significance value (Sig.) > 0.05 then the average value of the ethanol produced is the same, meaning that there is no significant effect of the addition of trace metals on the ethanol produced.
2. If the significance value (Sig.) < 0.05 then the average value of the ethanol produced is different, meaning that there is a significant effect of the addition of trace metals on the ethanol produced.

Table 5 ANOVA Test (output from the SPSS)

ANOVA

Ethanol Conc

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	151517,918	8	18939,740	7351,876	,000
Within Groups	46,371	18	2,576		
Total	151564,289	26			

The results of the ANOVA test can be seen in Table 5. Based on the ANOVA test, a significance value of 0.000 (Sig. < 0.05) was obtained, which means that there was a significant difference from the ethanol produced in each reactor. This shows that the addition of trace metals affects the ethanol produced.

4.3. Acidogenesis Product Formation

Another product produced during the anaerobic process is the formation of volatile fatty acids (VFA). The optimum conditions for ethanol formation were chosen to study the effect of adding trace elements to the formation of other acidogenesis products. During anaerobic processes, volatile fatty acids such as acetate, propionate, butyrate, and valeric are produced with a concentration higher than ethanol concentration. The dominant of VFA's species were acetate, with the concentration of about 4972.57 mg COD/L in the reactor with the addition Ni 0.5 mg/L and Mo 1 mg/L. The formation of VFA's is shown in.

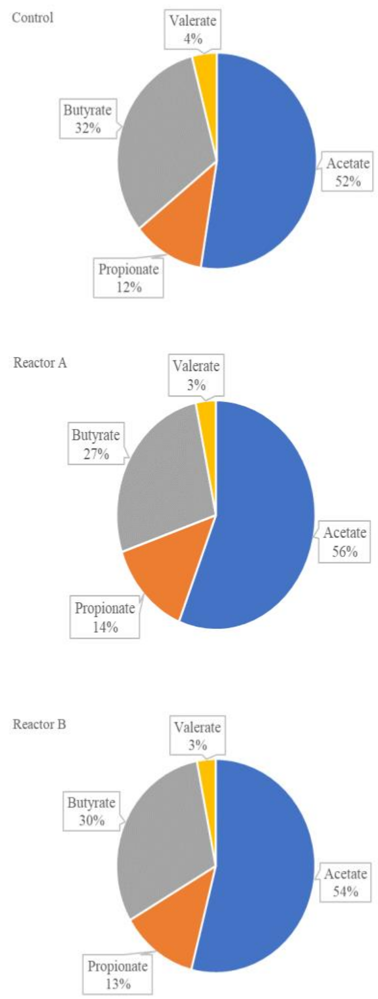

Fig. 4 Volatile fatty acids produced during the process

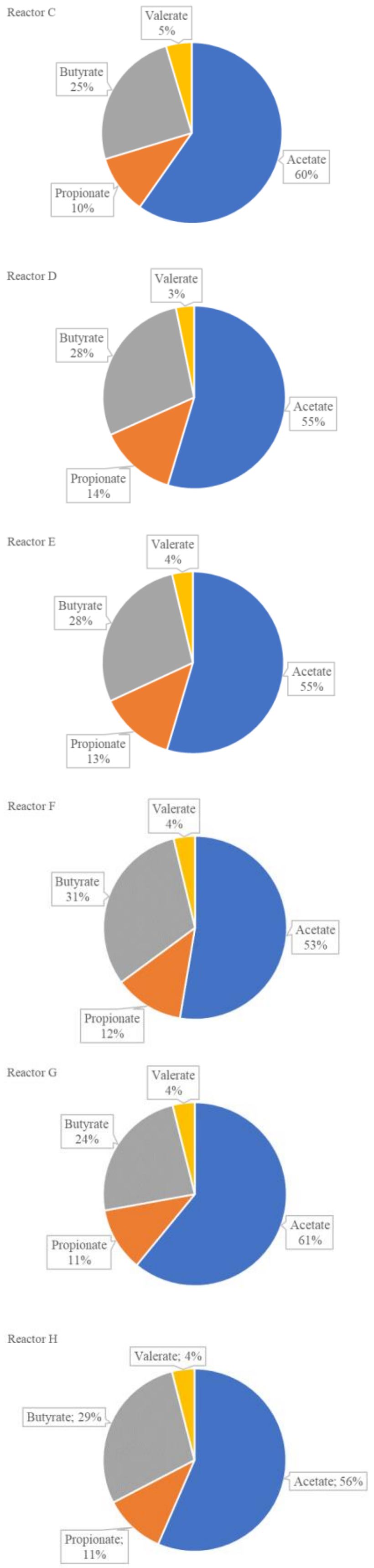

Fig. 5 Volatile fatty acids produced during the process (cont.)

The presence of trace elements can increase the optimization of the reaction rate at the hydrolysis and acidogenesis stages so that the production of organic acids will increase [29]. In other words, hydrolysis and acidogenesis bacteria got the benefit from the presence of nickel and molybdenum. In a study conducted by Evranos and Demirel [30], it was found that the presence of Ni can increase the performance of the hydrogenase enzyme and can increase bacterial growth, meanwhile, the presence of Mo can only increase bacterial growth.

Table 6 Anaerobic product formation during anaerobic processes

Parameter	Control	Reactor A	Reactor B
Ethanol	320.52	416.78	399.39
Acetate	3815.73	4315.81	4118.81
Propionate	844.81	1056.53	962.12
Butyrate	2305.08	2073.49	2319.98
Valerate	291.66	251.66	231.83
VSS	4296	4222	4192
COD (aq)	16520	14368	14886
pH	4.79	5.13	5.66

Parameter	Reactor C	Reactor D	Reactor E
Ethanol	480.11	510.21	578.44
Acetate	4961.03	4228.45	4199.52
Propionate	878.25	1056.62	1037.44
Butyrate	2078.94	2202.60	2168.87
Valerate	382.51	248.33	283.61
VSS	4371	4491	4264
COD (aq)	14953	14119	14368
pH	4.98	5.19	5.28

Parameter	Reactor F	Reactor G	Reactor H
Ethanol	511.24	521.68	520.88
Acetate	3819.33	4972.57	4281.65
Propionate	886.73	916.28	821.73
Butyrate	2274.72	1954.31	2175.13
Valerate	272.62	318.5	300.25
VSS	4517	4291	4028
COD (aq)	15027	14892	15120
pH	5.21	4.89	5.36

Note: units in mgCOD/L; VSS and soluble COD (COD aq) in mg/L; pH without unit.

In this study, the addition of Mo did not have much effect on the growth of biomass, this can be seen from the VSS concentration between the control and the addition of Mo and Ni did not have much impact on increasing the VSS concentration. The products produced in the anaerobic processing of POME are shown in Table 6.

The addition of micronutrients (Ni and Mo) can shift the product metabolism pathway towards the formation of ethanol. To see how many products in the form of ethanol and volatile acids produced can be compared using the value of the degree of acidification (DA) which is influenced by acetate, propionate, butyrate, and valerate; and degree of ethanoficiation (DE) [31]. The DA value is a parameter that can be used to evaluate the potential for acidogenesis for the formation of volatile acids. DA was obtained from the total volatile acid produced divided by the influent dissolved COD concentration, while DE was obtained from the ethanol concentration produced divided by the influent COD concentration [32]. The experimental results show that the highest DE value occurs in the reactor with the addition of 0.25 mg/L-Ni + 3 mg/L-Mo, which is 0.031. In other reactors, the DE value ranged from 0.017 - 0.028. The DE value in the control reactor is the smallest compared to other reactors, which means that the ethanol produced is getting smaller. Meanwhile, the DA value is formed between 0.394 - 0.450 with the smallest value occurring in the control reactor. As for the control reactor, the amount of ethanol and TVFA's produced is less than the reactor with the addition of Mo and Ni. In other words, the addition of Mo and Ni ions added to the reactor can affect the resulting acidogenesis product.

5. CONCLUSION

The results showed that the presence of the micronutrients, such as nickel (Ni) and molybdenum (Mo) had a significant effect on ethanol formation. The control variation without the addition of Ni and Mo resulted in ethanol of 320.52 mgCOD/L. The concentration of 0.25 mg/L Ni and 3 mg/L Mo gave the highest ethanol production about 578.44 mgCOD/L. Based on the results of this study, the addition of nickel (Ni) and molybdenum (Mo) can increase the formation of ethanol greater than the formation of ethanol without the addition of metal ions.

6. ACKNOWLEDGEMENT

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